



The effect of biological microreserves in a highly anthropized environment on the biology of *Natrix maura* (Linnaeus, 1758)

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Abstract.—Human disturbance in highly anthropized areas is known to have many negative effects on biodiversity. Due to their close relation to the environment (e.g., thermoregulation) and their limited movement capacities, reptiles (and snakes in particular) are excellent bioindicators of environmental quality. In addition, they carry out essential ecological functions, acting as prey and predator simultaneously. However, studies focusing on the effects that conservation efforts have on their populations after implementation are scarce. Hence, the aim of this study was to evaluate the effect of small protected areas on one of the most common snake species in the Iberian Peninsula, *Natrix maura*. During 2015 and 2018, samplings were conducted at eight different points in the Albufera de Valencia Natural Park in Spain, three of them being biological reserves. The results show that such protected areas have positive effects on the *N. maura* populations at different levels. In protected areas, population structures are more complex and the body condition of large individuals is better. The study also examined predatory pressure, but did not find a significant correlation between the abundance of predatory birds (herons) and injured or “scared” individuals of *N. maura*. In conclusion, such areas can be essential for allowing snakes to maintain their biological cycles, and in some cases even to prevent their disappearance from highly anthropized environments. Therefore, we strongly recommend the creation of more protected areas in order to promote the conservation of biodiversity in these areas.

Keywords. Conservation, ecology, human disturbance, snakes, Spain, wetlands

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Introduction

In a more and more anthropized world, the conservation of biodiversity is becoming an increasingly important challenge. However, there is an imbalance in the partitioning of conservation efforts among different animal groups (Czech et al. 1998; Gerber 2016). Especially in society in general, poorly accepted groups tend to receive less attention when planning and carrying out conservation and management projects (Czech et al. 1998). One group where this result is most evident is snakes, as their image is affected very negatively by many cultural factors, such as religious beliefs and the general media, leading to a learned fear and aversion (Ballouard et al. 2013). However, acting as predators and prey simultaneously, snakes are of vital importance to the function of many ecosystems. Moreover, given their tight relationship with environmental conditions and their limited movement capacities, snakes represent an ideal group for use as bioindicators of environmental quality

and for evaluating the success of conservation measures already implemented (Beaupre and Douglas 2009).

Natrix maura is one of the most common snake species of the Iberian fauna, and clearly the most abundant snake in water-related habitats (Pleguezuelos et al. 2002). In addition, a great ecological plasticity has allowed this species to persist in highly human disturbed areas, such as agricultural areas (Santos 2015). However, there is still a lack of knowledge regarding the effect of human disturbances on this species. This is concerning, as in areas where its population status had been studied previously, very negative effects of human activity on *N. maura* populations have been detected (Miras et al. 2009; Santos and Llorente 2009; Santos 2015).

The threats *N. maura* populations are facing are diverse. In addition to natural factors which negatively affect *N. maura* populations, including the improvement of natural predator populations such as herons (e.g., Garrido et al. 2012), the great majority of the threats are related to human activity. Road mortality is an important

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Figure 1. Satellite image of the study area, the Albufera de Valencia Natural Park, obtained from Google Maps (accessed: 25 January 2019), indicating the locations of the eight sampling points.

factor, especially in wetlands, as roads usually run parallel to water channels (Llorente et al. 2005; Santos 2015). In addition, the destruction, fragmentation, and disturbance of *N. maura* habitats are important threats (Santos et al. 2002) and, given that it is a predator, the reduction of prey availability has also negative effects on the species (Filippakopoulou et al. 2014). The massive use of plant protection products in agriculture and contaminants being dumped into rivers from urbanization have noticeably reduced the water quality in this species' habitats. Given that the prey items of *N. maura* are mainly aquatic, studies have shown that *N. maura* has a great capacity for the bioaccumulation of contaminants (Santos et al. 1999). In addition, ecological alterations of the ecosystems by the introduction of exotic species has also exerted direct or indirect negative effects on *N. maura* populations (e.g., Alarcos et al. 2009). Finally, the fear and negative image that ophidians have in the general population leads to their encounters with humans often ending with the snakes' death, even for those that are absolutely inoffensive (Hailey and Davies 1987). The effects of all these factors were seen, for example, in the Ebro Delta of Spain, when Santos and Llorente (2009) detected a dramatic reduction in the abundance of *N. maura* in a span of only 13 years.

As in the Ebro Delta, in the Albufera de Valencia Natural Park (Spain) the use of the major terrain is for rice plantations (Sánchez 2008). This intensive agriculture has promoted the massive use of fertilizers, herbicides, and insecticides, which in the past decades has led to massive

eutrophication problems and a drastic decrease in general water quality (e.g., Soria 2006). To confront this problem, several natural reserves were created between 2006 and 2011 in order to foment biological conservation and the recovery of water quality. These reserves are former rice fields which were transformed into artificial wetlands, recovering the marshy habitats and the associated plant and animal biodiversity, and restoring (at least in small areas) the natural state of the Albufera wetland. The importance of this type of biological reserve has already been reported for various groups of organisms, including birds (Rodrigo et al. 2018; Sebastián-González et al. 2013), amphibians (Reques 2004), and turtles (Drechsler et al. 2018).

Fauna conservation studies often focus on “umbrella” or “indicator” species or zoological groups, for example birds. However, this implies a simplification of the ecological relationships and has already been criticized, as the conservation of one group does not necessarily entail the conservation of the others (e.g., Figuerola and Green 2003). In the case of reptiles (with the exception of turtles with conservation problems), there are very few studies which evaluate the effects of conservation measures on their target populations (Shine et al. 1998; Webb et al. 2005; Schoemaker 2007).

The aim of this study is to evaluate the effect of small biological reserves in highly anthropized environments on several aspects of the biology of *N. maura*, especially population dynamics. Given that this species is the most common reptile in aquatic environments in the Iberian Peninsula, these data will be of great use when comparing biological quality of coastal wetlands throughout the Iberian Peninsula, and at the same time, for evaluating the success of conservation measures that have been taken.

Materials and Methods

Study Area

The study area is the Albufera de Valencia Natural Park, located about 10 km south of Valencia city in Spain (39°21'18.7"N 0°21'38.4"W). It is one of the most important wetlands of the Iberian Peninsula, with an area of ~21,000 ha. Approximately 3,000 ha correspond to the central lagoon, while the remaining 18,000 ha mainly correspond to intensive rice fields (*Oryza sativa*) [Fig. 1]. The water supply of this environment has several different origins, including the Júcar river, rain water from highly urbanized watersheds, springs from subterranean aquifers, urban or industrial effluents (depurated wastewater), and irrigation returns from the agriculture in the area. The study area is highly anthropized for the agricultural use, so that the natural marsh vegetation is restricted to canals and the perimeter of the central lagoon, and is dominated by Common Reed (*Phragmites australis*) and Southern Cattail (*Thypha dominguensis*).



Figure 2. Satellite images of the three natural reserves: Pipa (A), Milia (B), and Illa (C), obtained from Google Maps (accessed: 5 December 2019).

Embedded in this anthropized environment are the three studied biological reserves: Tancat de la Pipa, Tancat de Milia, and Tancat de Illa (Fig. 2). All three reserves are former rice fields that have been transformed into artificial wetlands in order to restore water quality, foment the conservation of biodiversity, and provide areas for environmental education. The access to these areas is restricted to the fulfilment of these specific objectives. The Tancat de la Pipa (Pipa) is the oldest and largest reserve, created in 2006 with an area of about 40 ha, and different types of shallow marshy habitats have been recreated there, including canals with dense vegetation on the shores and permanent lagoons. The other two reserves were created in 2011 and are characterized by more active management of the water levels and vegetation. The Tancat de Milia (Milia) has an intermediate area of 33.4 ha and the Tancat de Illa (Illa) is the smallest, covering only 16 ha. In addition to the reserves, five additional points were sampled: (i) the Catarroja port and the surrounding rice fields (AR), an area far away from the central lagoon and high anthropic pressure due to the presence of bars, frequently used roads, fishing, and boat traffic; (ii) rice fields near the Silla port (LR), an area close to the central lagoon and with less anthropic pressure; (iii) the surroundings of El Palmar (Palmar), an area with a relatively high anthropic pressure, abundant vehicle traffic, and fishing activity; (iv) Socarrada (SC), an area close to Illa; and (v) the Muntanyeta dels Sants (MS), a small urbanized area embedded in the rice field environment (Fig. 1).

Fieldwork

Between March and November in both 2015 and 2018, one researcher carried out random searches for *N. maura* for 1.5–2 hours at the eight sampling points. All censuses were carried out by the same researcher and were initiated 2–3 hours after sunrise. The sampling was repeated at each point every two weeks in 2015 and once per week in 2018. In each survey, adverse weather conditions were annotated, such as clouds, wind, or rain. All individuals of *N. maura* observed were recorded, indicating whether

they were adults or juveniles when possible. Individuals were considered adults based on SVL > 250 mm (males) and SVL > 300 mm (females) [Santos 2015]. In parallel to the counts, whenever possible, all individuals were captured by hand and placed in individual cloth bags until posterior measurements could be taken. Once the censuses were finished, the captured individuals were processed, measuring basic biometry with the use of a measuring tape and a weighing scale: snout-vent length (SVL, to the nearest 0.5 mm), total length (to the nearest 0.5 mm), and weight (with a precision of 0.01 g). In addition, records were made of the presence of injuries, scars, or a broken tail, which could be interpreted as the result of a predation event, or if there were gut contents (these individuals were excluded from body condition analysis). Each individual was marked with a unique code of small notches on the ventral scales in order to control for recaptures (Lang 1992). After measurements were taken, each individual was released at the capture point.

Data Analysis

To determine the abundance, the numbers of individuals observed were standardized for the duration of each census (in hours), obtaining the values in individuals/hour. Data were grouped by areas, years, seasons (as “Spring” for March–May, “Summer” for June–August, and “Autumn” for September–November), whether the area was protected or not, and whether it was close to the central lagoon (<2 km) or far from the lagoon (> 2 km). In order to determine statistical significance, a Poisson Logit Linear Regression Model (Poisson GLM) was performed. Effects of the presence of clouds or other adverse meteorological conditions on the snake censuses were eliminated by comparing the snake counts on the days with such conditions with the days immediately before and after which had favorable conditions (Poisson GLM, $z = 1.043$, $p = 0.297$).

In order to analyze predation risk, a Binomial Logit Linear Regression Model was performed considering the following variables: SVL, Type of dorsal coloration (zig-

zag vs. bilineata, Santos et al. 2017), Sex, Year, and Site. For this analysis, individuals with SVL < 250 mm ($n = 305$) were excluded, because large individuals are known to be more prone to predation (Santos et al. 2011). In addition, in the case of the reserves, the proportions of injured individuals were calculated (Santos et al. 2011), and these data were compared with the abundances of birds that are potential predators of *N. maura*, especially large herons (Great Egret, *Egretta alba*; Grey Heron, *Ardea cinerea*; and Purple Heron, *Ardea purpurea*). The number of birds was obtained by conducting weekly counts during the same period as each snake census. The bird counts were accomplished with binoculars and telescopes, always during the first four hours after sunrise and avoiding adverse meteorological conditions such as wind, rain, or fog. In the cases of Pipa and Illa, these counts were made on foot, recording all individuals seen; while in the case of Milia, with less vegetation surrounding open water areas, counts were made from inside a parked car.

Data from both years were used for constructing the population pyramids, without discriminating periods. The captured individuals for each area were grouped in intervals of 150 mm SVL and the proportions of individuals corresponding to each interval were calculated with respect to the total area. A chi-square test was performed to determine statistically significant differences.

Finally, to study the body condition, the relation between weight (g) and size (SVL, in mm) of the individuals was represented and the tendency line was drawn. Individuals with an SVL above 600 mm were excluded, as such individuals appeared in only two areas (Pipa and Milia). The tendency line of the global data cloud served as the reference function. Afterwards, the data were separated by areas and the correlation coefficient (R) and determination coefficient (R^2) of the corresponding data cloud were calculated with the reference function. A low coefficient for an area indicates that the gain of weight of the individuals in this area does not follow the mean increase of the general population. In order to analyze the evolution of body condition in the different areas in greater detail, the SVL and weight values were log-transformed and the residuals of the correlation between these two variables were calculated. To determine statistical differences between protected and non-protected areas for the residuals, Kuskal-Wallis tests were performed for each SVL interval.

Results

A total of 721 snakes was recorded in 459 sampled hours. The abundance analysis showed an almost significant reduction of abundance values between 2015 (1.76 ± 1.80 ind/h) and 2018 (1.45 ± 1.55 ind/h) [Table 1]. This decrease is especially evident in Illa for both adults and juveniles and in AR for adults (Fig. 3). In addition, areas

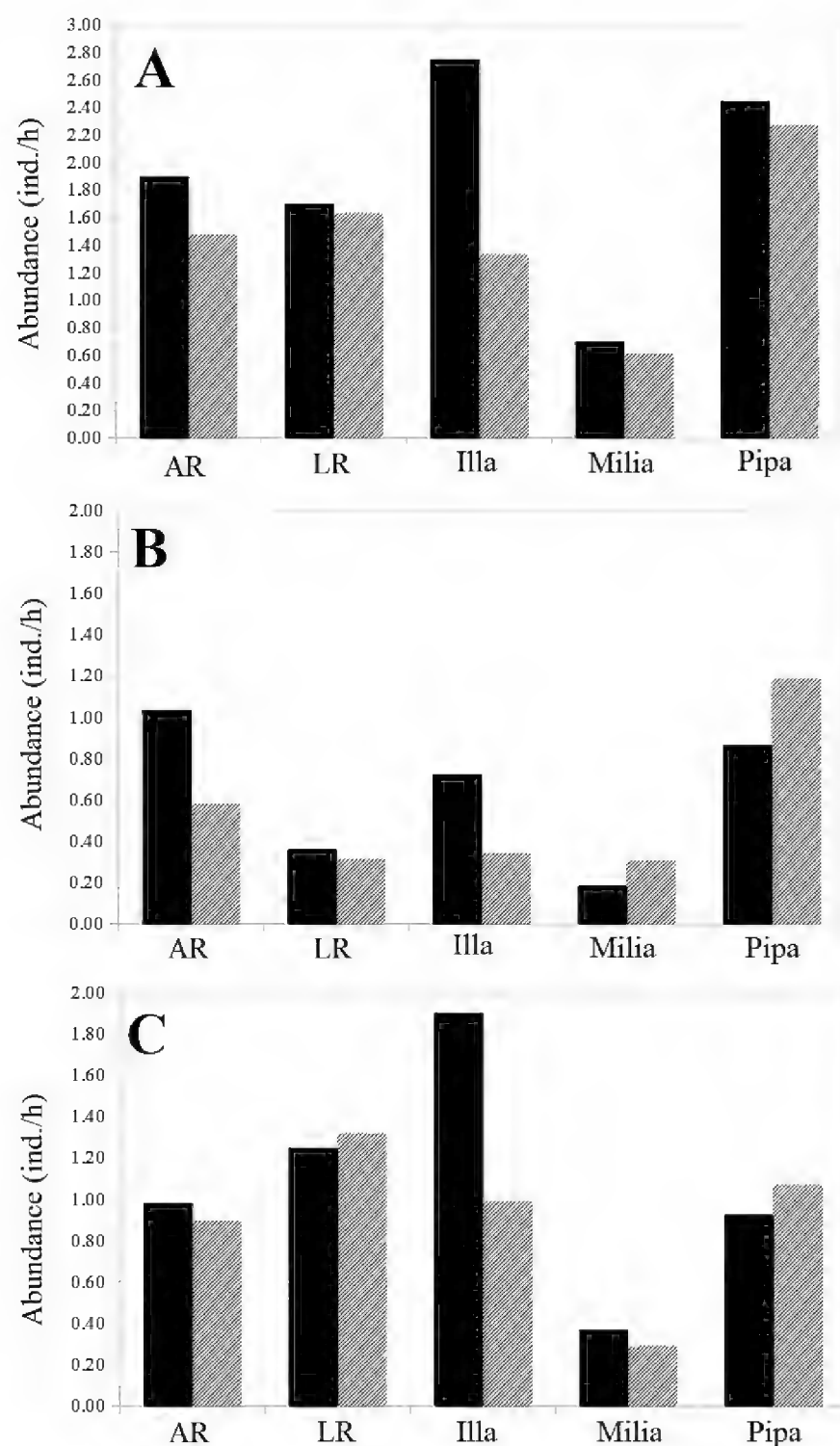


Figure 3. Abundances of *Natrix maura* in the different study areas in 2015 (filled bars) and 2018 (striped bars), for the total population (A), adults only (B), and juveniles only (C).

located near the central lagoon and in protected areas showed abundances which seem to be significantly lower than in areas far from the lagoon and non-protected areas, respectively (Table 1).

Significant differences were found in the distributions of sizes between areas ($\chi^2 = 362.2$, $df = 35$, $p < 0.001$). The population pyramids (Fig. 4) show that the most mature populations, i.e., those with the highest proportions of large individuals, correspond to Pipa and Milia, which presented ~10–15% of individuals above 600 mm SVL. In the other areas, the populations are mainly formed

Table 1. Results of the GLM for Poisson distributions evaluating the effect on the abundance of *N. maura* of five different variables: Year, Season, Area, Protected/non-protected and Close/far from the central lagoon.

| Variable | Coefficient estimate | z | p |
|-------------------------|----------------------|--------|--------|
| Year | -0.318 | -1.937 | 0.053 |
| Season | -0.122 | -1.329 | 0.184 |
| Area | 0.315 | 3.843 | <0.001 |
| Protected/non-protected | -0.775 | -3.364 | <0.001 |
| Close/far from lagoon | 0.784 | 3.835 | <0.001 |

Table 2. Values of the correlation and determination coefficients for the increase of weight with body size with respect to the reference function for each sampling area.

| Sampling area | Correlation coefficient (<i>R</i>) | Determination coefficient (<i>R</i> ²) |
|---------------|--------------------------------------|---|
| SC | 0.982 | 0.965 |
| LR | 0.953 | 0.908 |
| AR | 0.950 | 0.903 |
| Pipa | 0.942 | 0.888 |
| Palmar | 0.939 | 0.881 |
| Milia | 0.929 | 0.863 |
| Illa | 0.888 | 0.789 |
| MS | 0.886 | 0.785 |

by immature individuals, specifically individuals with 150–299 mm SVL. The second most abundant group had 300–449 mm SVL, and is formed by young adults. In these areas, very few individuals (~5%) reached an SVL above 450 mm.

The results obtained regarding predation indicate that none of the variables considered significantly affected predation risk ($z = 1.929$, $p = 0.054$, for SVL; $z = -0.774$, $p = 0.439$, for Coloration; $z = -1.782$, $p = 0.075$, for Year; $z = -0.326$, $p = 0.744$, for Sex; and $z = -0.857$, $p = 0.392$, for Site). Significant correlations were not found between either the abundance of potential predator birds (expressed as number of birds detected per census and ha of the reserve) or the proportion of injured *N. maura* individuals (Lineal Regression Model, $F_{3,10} = 4.424$, $p = 0.126$, considering only the three mentioned large heron species; and Lineal Regression Model, $F_{3,10} = 2.757$, $p = 0.195$ for all heron species).

The analysis of body condition showed that in the sampled population of *N. maura* in the Albufera de Valencia Natural Park, weight (*W*, in g) varies with body size (SVL, in mm) according to the following function ($n = 649$):

$$W = 0.787328 e^{0.009809 SVL} \quad (R^2 = 0.95)$$

The comparison of the data clouds of each area with this reference function showed that MS and Illa were the areas with the lowest correlation coefficients for these variables, and SC and LR were the areas with the highest correlation values (Table 2). Among the biological reserves, Illa was the least correlated and Pipa the most correlated. Comparing protected areas with non-protected areas, a clear difference was seen regarding body condition, with large individuals (> 450 mm SVL) presenting significantly higher body

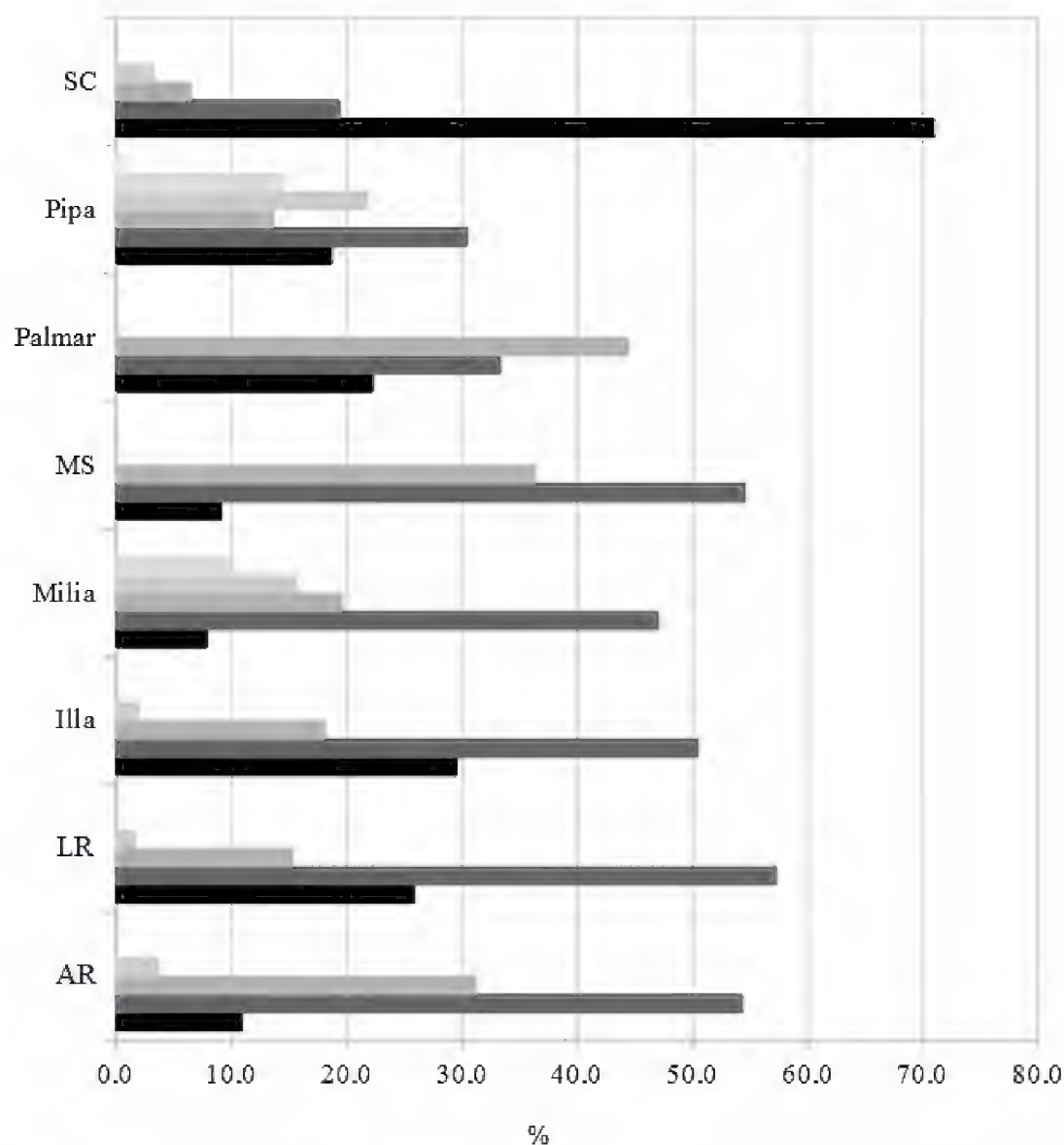


Figure 4. Population pyramids of each area representing the proportion of individuals for each SVL interval in the population: < 150 mm (■); 150–299 mm (■); 300–449 mm (■); 450–599 mm (■); 600–749 mm (■); and >750 mm (■).

Table 3. Sample size (in parentheses), and values of mean \pm standard deviation of weight (in g) for the individuals in different body size ranges for each area, comparing the protected (Illa, Milia, and Pipa) and non-protected areas (AR, LR, MS, Palmar, and SC); and the results of the Kruskal-Wallis test comparing the residuals of the correlations between the log-transformed SVL and weight data from protected and non-protected areas.

| | SVL (mm) | | | |
|---|------------------------|--------------------------|-------------------------|-------------------------|
| | < 150 | 150–299 | 300–449 | 450–599 |
| SC | (22) 2.31 \pm 0.51 | (6) 3.67 \pm 0.97 | (2) 26.50 \pm 4.38 | (1) 80.80 \pm 0.00 |
| LR | (31) 2.74 \pm 0.55 | (71) 6.68 \pm 5.12 | (19) 31.51 \pm 10.98 | (2) 91.92 \pm 54.28 |
| AR | (15) 2.51 \pm 0.33 | (72) 9.50 \pm 6.04 | (42) 30.04 \pm 10.34 | (5) 60.28 \pm 9.75 |
| Pipa | (41) 2.53 \pm 0.51 | (67) 6.45 \pm 4.68 | (28) 34.06 \pm 14.15 | (44) 131.70 \pm 43.04 |
| Palmar | (5) 2.96 \pm 0.61 | (9) 11.09 \pm 7.49 | (12) 37.57 \pm 21.26 | - |
| Milia | (4) 2.39 \pm 0.14 | (24) 8.16 \pm 5.84 | (8) 30.08 \pm 7.81 | (5) 100.71 \pm 35.15 |
| Illa | (31) 2.64 \pm 0.64 | (53) 6.92 \pm 4.98 | (19) 35.47 \pm 14.49 | (2) 91.17 \pm 18.17 |
| MS | (1) 2.30 \pm 0.00 | (6) 15.39 \pm 7.48 | (4) 30.64 \pm 4.85 | - |
| Protected | (76) 2.57 \pm 0.56 | (144) 6.91 \pm 5.00 | (55) 33.97 \pm 13.45 | (51) 127.14 \pm 42.88 |
| Non-protected | (74) 2.57 \pm 0.53 | (164) 8.38 \pm 6.05 | (79) 31.48 \pm 12.51 | (8) 70.75 \pm 26.38 |
| Kruskal-Wallis results for residuals | | | | |
| Mean (Protected) | (76) 0.047 \pm 0.224 | (144) -0.045 \pm 0.211 | (55) -0.081 \pm 0.225 | (51) 0.109 \pm 0.262 |
| Mean (Non-protected) | (74) 0.099 \pm 0.213 | (164) -0.011 \pm 0.225 | (79) -0.103 \pm 0.167 | (8) -0.164 \pm 0.163 |
| DF | 1 | 1 | 1 | 1 |
| X² | 3.102 | 1.782 | 0.056 | 7.177 |
| P | 0.078 | 0.182 | 0.812 | 0.007 |

condition values in protected areas than in non-protected areas (Table 3).

Discussion

The results reported here show that biological reserves are local areas of importance for the conservation of snakes in the Albufera de Valencia Natural Park. The population structure in protected areas is more complex, individuals reach larger body sizes, and their body condition is better in comparison to non-protected areas. The effect of such reserves as natural environments can noticeably reduce some threats and pressures on snakes, such as agricultural activities and human presence, which impart higher mortality (due to machinery, roadkill, or aggression caused by the aversion to snakes in many people) [e.g., Whitaker and Shine 2000]. It is precisely expected that this mortality is accentuated in larger individuals (Shine and Koenig 2001). Moreover, in non-protected areas, the low environmental quality and human disturbance also have been shown to negatively affect the availability of prey, such as fish and amphibians (Lawler 2001; Marco 2002). Finally, it has been demonstrated that the bioaccumulation rate of toxics in *N. maura* is more important in large individuals than in small ones (Santos et al. 1999; Lemaire et al. 2018). While not directly measured here, all these factors could play synergistic roles in explaining the results of this study. Although a decrease in abundance was found in protected areas in relation to non-protected ones, this could be related to differences in detectability (e.g., dense vegetation in reserves, abundant junk items serving as hiding spots in anthropized areas, etc.). This could also explain the differences between areas close to and far from the

lagoon, as the latter tended to be more anthropized.

The results of this study also indicate that characteristics of protected areas, such as size and management, could play important roles in their ability to conserve *N. maura* populations, as not all protected areas showed the same results. For instance, although Illa is a protected area, its reduced size and the fact that it is surrounded by a heavily-travelled road could prevent the development of a population as complex as the ones observed in Milia and Pipa. Another possible explanation could be the presence of predators, especially birds, and in particular herons. These birds also tend to congregate in the reserves, using it for reproduction, feeding, and as temporary roosts during migration (e.g., Gosálvez et al. 2012; Pérez-Granados et al. 2013). Many birds, especially herons, predate on *N. maura* (e.g., Amat and Herrera 1977; González and González-Solís 1990). In fact, the proportions of injured *N. maura* individuals were higher in Pipa and Milia than in the other areas. However, in this study we did not find evidence for a significant effect of bird density on the status of *N. maura* populations, and in most cases the correlations were even negative. This could be explained by the greater density of vegetation in the protected areas, providing the snakes with more refuges and possibilities for hiding and escaping from predators. We also have to take into account the effect of the “bias of the survivor,” as we can only see and measure what correspond to failed predation attempts (Gregory and Isaac 2005). Finally, the coloration of the snakes has been found to have a significant effect on predator attack rate, where in dense habitats individuals with a zig-zag pattern (Batesian mimicry) are attacked significantly less than individuals with striped dorsal patterns (Santos et al. 2017). However, this study did not detect a significant

effect of dorsal coloration on predation risk.

The results presented here do not allow for inferences on the long-term tendencies of the populations in the studied areas or the general population of *N. maura* in the Albufera de Valencia Natural Park. However, the fact that a significant decrease of snake abundance was detected between 2015 and 2018 can be interpreted as an indicator that the populations may be suffering a decline. In fact, snake populations in general are known to be declining considerably, and there are indications that in Europe the most probable causes are habitat deterioration, low prey availability, and pollution (Reading et al. 2010). In the case of the Iberian wetlands, industrialization, the modernization of sowing and harvesting techniques, the massive use of fertilizers, habitat loss and fragmentation, and other factors have already caused an important decrease in snake populations in the Ebro Delta (Santos and Llorente 2009), an area very similar to the area of this study. Specifically, the authors detected a decline in the Ebro Delta of about 50%, in some cases even 100%, of the *N. maura* populations in only 13 years. However, the lack of data for the Albufera de Valencia Natural Park on the status of the *N. maura* population in the past makes it impossible to confirm whether a similar situation is happening in the Albufera de Valencia Natural Park.

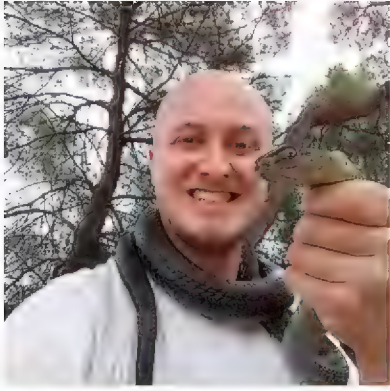
In conclusion, the existence of biological reserves in this highly anthropized environment has had a positive effect on *N. maura* populations on different levels. Considering that human disturbance and the effects of intensive agriculture (landscape homogenization, loss of boundaries, temporal disturbance, and use of chemical products) are growing stronger and that a general decline of snake populations has already been described, we could even affirm that such protected areas are essential for snakes to maintain their biological cycles and, in extreme cases, even to avoid the disappearance of populations of species like *N. maura* in this type of environment. Taking all of these considerations into account, it is evident that the creation of more such protected areas is important and has to be promoted in highly anthropized areas, with the aim of conserving and protecting snakes, a group of predators that is essential for the function of many ecosystems and to which it is increasingly necessary to pay attention.

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